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Request for grant of a patent

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1. Your reference

P21993/CPA/RMC

2. Patent application number
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9812376.3

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

The Queen's University of Belfast
8 Malone Road
BELFAST
BT9 5BN

Patents ADP number *(if you know it)*

If the applicant is a corporate body, give the country/state of its incorporation

4. Title of the invention

"Peptide"

5. Name of your agent *(if you have one)*

Murgitroyd & Company

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

373 Scotland Street
GLASGOW
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Patents ADP number *(if you know it)*

1198013

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Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

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Number of earlier application	Date of filing *(day / month / year)*

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a) any applicant named in part 3 is not an inventor, or
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Description	6
Claim(s)	
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Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application.

Signature Murgitroyd & Company Date 9 June 1998
Murgitroyd & Company

12. Name and daytime telephone number of person to contact in the United Kingdom: Roisin McNally, 0141 307 8400

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Notes

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"Peptide"

The present invention relates to a modified analogue of the signal peptide sequence from Karposi syndrome fibroblast growth factor (kFGF) to be used as a cell-permeant vehicle for the intracellular delivery of covalently linked anti-sense peptide nucleic acid sequences (PNAs).

PNAs have potential uses as antisense molecules for the control of gene expression. Since they are capable of binding tightly to DNA and RNA targets thus preventing DNA transcription to RNA and RNA translation to protein. These molecules thus have two potential uses of commercial importance:

1. As research reagents where scientists use antisense strategies to ablate selected genes in order to understand their function.

2. As pharmaceutical compounds for companies seeking to develop nucleic acid-based therapies.

Conventional anti-sense oligonucletide in vivo delivery is highly inefficient, even if long-lasting, less polar

phosphorothioates are used.

It is an object of the present invention to use cell permeable peptide import (CPPI) to deliver PNAs to live target cells.

Use of conventional oligonucleotides is being reduced due to the development of PNAs (Neilsen, et al., 1991), which are much more stable, being resistant to enzymic degradation (Jordan, et al., 1997). PNAs replace the phosphodiester backbone of nucleic acid with repeating N-(2-aminoethyl)glycine units to which natural nucleobases are attached through methylenecarbonyl linkers. Although more stable, PNAs suffer from similar accessibility problems as phosphorothioates do, and passive diffusion of unmodified PNA across lipid membranes is not efficient (Wittung, P., et al., 1995).

A small number of native peptide sequences can translocate across membranes of living cells in an energy-independent and receptor-independent manner. These peptides have been used to import active cargo into the cell. For example a peptide from the homeodomain of *Antennapedia* has been successfully used to import both peptidal inhibitors of protein kinase C (Theodore, et al., 1995) and conventional anti-sense oligonucleotides (Allinquant, et al., 1995).

The present invention provides use of cell permeable peptide import (CPPI) to deliver peptide nucleic acids (PNAs).

The present invention provides use of the signal peptide sequence from Karposi syndrome fibroblast growth factor (kFGF) for delivery of antisense peptide nucleic acid sequences (PNAs).

The invention provides modified peptide sequence I as detailed herein.

The invention also provides peptide sequences II and III as detailed herein.

The invention provides use of a peptide as defined herein together with lysine residues for multiple presentation of peptide nucleic acids.

The invention further provides use of peptides as defined herein together with lysine residues in the simultaneous presentation of different peptides nucleic acids.

The present invention combines the two above technologies to use CPPI to deliver PNAs to in vivo targets.

Example

In order to determine the best delivery system, a comparison of the ability of three different cell permeant peptides to accumulate in whole cells was undertaken. The three peptides (Table 1) were labelled with carboxyfluorescein and the amount accumulated intracellularly was assayed after exposure of cells to 50μM; peptide II = 0.4μM; peptide III = -0.4μM.

Table 1

I	CFI	A A V A L L P A V L L A L L A P K K K
II	CFI	R F A R K G A L R Q K N V H E V K N
III	CFI	R P R P Q Q F O G L M

r

Key Peptide I : modified kFGF signal sequence
Peptide II : PKC pseudosubstrate sequence
Peptide III : modified substance P
CFI : Carboxyfluorescein
Or : Ornithine
Boldface : Modifications to original sequence

Peptide I was modified to contain three lysines C-terminal of the hydrophobic signal sequence. This peptide, therefore, can accommodate three PNAs, each bonded to a lysine epsilon amino group. This can be extended using the Multiple Antigen Presentation (MAP) technology to present eight (or more) PNA's on one peptide I sequence. A 'lysine tree' constructed in this way accommodates eight copies of the same PNA (see Fig 1A), thus increasing the effective concentration delivered by each CPPI. Alternatively a carrier can be constructed containing three (or more) different PNAs directed towards different sites on the same target mRNA (see Fig. 1B). This strategy has been termed 'molecular triangulation' (Branch, A.D., 1998).

Fig. 1A - Multiple presentation of a single PNA species

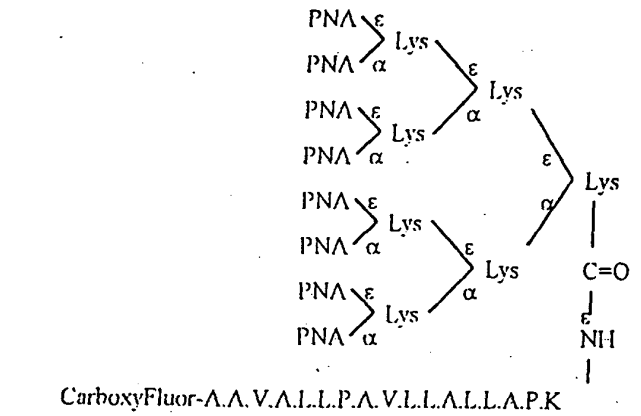
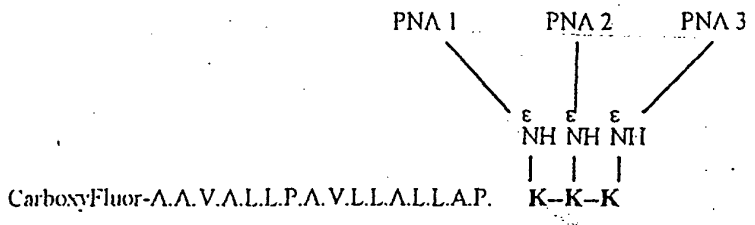


Fig. 1B - Simultaneous presentation of 3 PNAs directed to different sites on same target RNA



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